APPLICATION OF SULPHATE REDUCING - SULPHIDE OXIDISING BACTERIAL SYMBIOSIS FOR WASTEWATER TREATMENT

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MASTER OF TECHNOLOGY

by

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to the

DEPARTEMENT OF CIVIL ENGINEERING

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CERTIFICATE

It is certified that the work contained in the thesis entitled "*Application of Sulphate Reducing - Sulphide Oxidising Bacterial Symbiosis for Wastewater Treatment*" by Mr Sabumon P.C., has been carried out under my supervision.

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ABSTRACT

This investigation is attempted to advance the state of the art of the process which utilises the symbiotic relationship between the Sulphate Reducing Bacteria (SRB) and Sulphide Oxidising Bacteria (SOB) for degradation of organic matter present in wastewater. The major emphasis has been on the developement of the desired microbial system without any external seed and comparative evaluation of the two types of Multistage Reversingflow Bioreactor (MRB) systems in which biological vessels (BVs) simulate conditions corresponding to configurations described as upIfow sludge blanket and stationary fixed film Two bench scale models, one designed to achieve self granulation of sludge (SGS), and the second designed to promote growth of SRB/SOB on additional nonreactive surface, were set up and operated over a period of four months. Domestic wastewater supplimented with organic matter from sugar cane molasses was used as feed to develop the desired microbial population. Several visual and microscopic observations confirm the presence of significant numbers of SRB and SOB in all the biological vessels.

Results indicated that it is possible to develop SGS and microbial population of SRB/SOB which could attach to the nonreactive surface without any external seeding Domestic wastewater could serve as a source of these organisms. Immobilised growth conditions and suspended growth conditions in BVs yield similar results in terms of organic matter utilisation. However, it is felt that immobilised growth system would have yielded much better results if proper plug flow conditions were ensured in BVs Consumption of sulphate appears to be insignificant and hence several types of wastewaters could be treated using MRB systems MRB system efficiency appears to be comparable to that of activated sludge process Considering the reported merits of MRB, it appears to be promising alternative to conventional (albiet modified) biological processes for wastewater treatment.

KEY WORDS

Wastewater Treatment, Biological Processes, Aerobic-Anaerobic Processes, Dispersed Growth, Immobilised Growth, Multistage Reversingflow Bioreactors, Sulphate Reducing Bacteria, Sulphide Oxidising Bacteria, Self Granulated Sludge, SRB-SOB Symbiosis, Sulphate Reduction, Sulphide Oxidation.

INTRODUCTION

Biological processes have been and will continue to be one of the most economical means of treatment of wastes and are also compatible with nature's way of assimilating the pollutants Biological wastewater treatment processes can be classified into two broad categories viz. aerobic and anaerobic from the aspect of final electron acceptors in degradation of organic matter. These two processes have several advantages and disadvantages when compared with each other. For example, the aerobic treatment processes produce better effluent quality in shorter reaction time, but require more energy and produce more excess sludge The advantages of the aerobic treatment become the disadvantages of the anaerobic treatment and vice versa

Several technological advances in the field of both aerobic and anaerobic treatment have significantly improved process operations and economics. Most of the process improvements have come from changes in engineering aspects rather than fundamental shifts in the underlying microbial principles. Since most of the disadvantages of the aerobic and anaerobic processes come from the microbiological principles, it is very difficult to expect radical changes as long as we continue to employ microbial cultures normally associated with the conventional (albiet modified) aerobic-anaerobic processes. On the other hand, there would be a good possibility to establish new wastewater treatment processes, if we can introduce microbial cultures distinctly different from those employed in conventional aerobic and anaerobic processes. In the present research, an attempt is made to explore the possibility of employing a microbial culture quite distinct from conventional aerobic and anaerobic processes as reported in recent literature (Takahashi and Kyosai, 1988). A Multistage Reversingflow Bioreactor (MRB) is a relatively new wastewater treatment process developed in Japan (Takahashi and Kyosai, 1988) based on symbiotic interaction between sulphate reducing bacteria and sulphide oxidising bacteria The following section reviews the underlying microbial principles involved and analyses the suggested engineering approach for treatment of wastewater

LITERATURE REVIEW

General

MRB controls the interaction between sulphate reducing bacteria (SRB) and microaerophilic sulphur oxidising bacteria (SOB), resulting in self granulated pellet formation. The developers of MRB have claimed several advantages of the process over the conventional aerobic biological wastewater treatment, which makes the process attractive and worth evaluating. The advantages include lower energy requirement (low oxygen supply and no recycling of sludge), less sludge production (low sludge yield by SRB and anaerobic bacteria) and no necessity for a final settling tank (sludge blanket formed in the biological reactor acts as a filter for suspended solids)

MRB Process Description

Fig. 1 is a conceptual representation of MRB Influent is introduced into the first downflow aeration vessel (AV). Suspended solids are not allowed to accumulate in AVs. The next vessel is a biological reaction vessel (BV) in upflow mode without aeration. Suspended solids with settling velocity higher than the upflow velocity accumulate in this vessel. The following vessels are in the same sequence. Each stage consists of an AV and a BV. There may be several stages. The AV is for oxygen supply only. In the BV, suspended solids and biomass are gently mixed by the upflow resulting in formation of Self Granulated Sludge (SGS).

Important Microbial Species Involved

The microbial population involved in a MRB system consists of a wide variety of microbes including those present in conventional aerobic and anaerobic processes However, two types of micro organisms namely, sulphate reducing bacteria (SRB) and microaerophilic sulphide oxidising bacteria (SOB) play a significant role Hence relevant details of these two types of organisms are presented as follows

Sulphur bacteria: The bacteria that oxidise or reduce significant amounts of organic sulphur compounds exhibit a wide diversity of morphological and biochemical characteristics One group of sulphate reducing bacteria, consists of single cell forms that grow anaerobically and reduce sulphate, SO_4^{2-}, to hydrogen sulphide, H_2S Another group, aerobic sulphur oxidisers, oxidise reduced sulphur compounds aerobically to obtain energy for chemoautotrophic growth. The sulphur bacteria of most importance in the water and wastewater field are the sulphate reducing

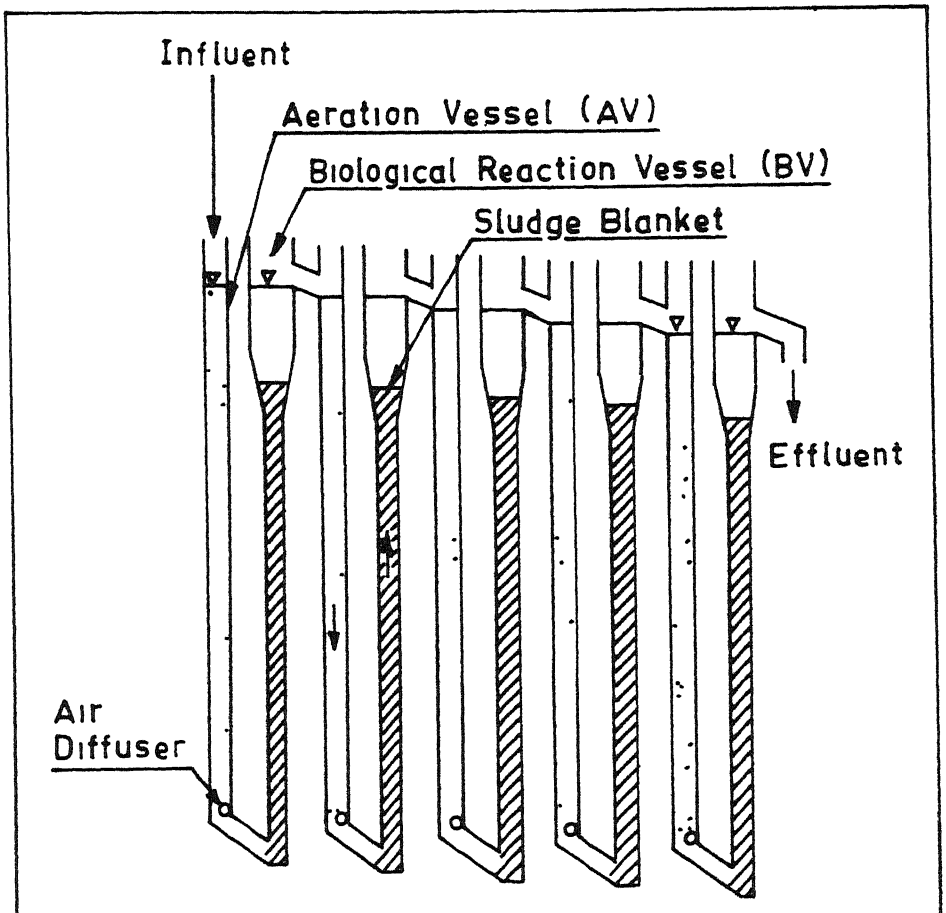


Fig. 1. Flow Sheet of MRB Reactor (Vertical Cross Section)

bacteria which include *Desulphovibrio*, and the single celled aerobic sulphur oxidisers of the genus *Thiobacillus* (APHA, 1985)

Beggiatoa and *Thiovulum* species are representatives of the gradient-type colourless sulphur bacteria which live in aquatic environments at the transition between oxygen and hydrogen sulphide (Jorgensen and Revsbech, 1983). They are often observed as isolated patches on organic-rich sediments along protected shores of lakes and of the sea. They may also cover the decaying remains of dead animals and plants from which large amounts of H_2S are produced. In the presence of oxygen, the bacteria oxidise H_2S to elemental sulphur, S^o, which accumulates as sulphur droplets inside the cell wall The elemental sulphur can be further oxidised to sulphate by *Beggiatoa* species. Under anaerobic conditions, S^o may serve as an alternative electron acceptor in *Beggiatoa* species and be reduced back to H_2S (Nelson and Castenholz, 1981).

When oxygen and sulphur coexist, they react spontaneously with a half life in the order of one hour. The sulphur bacteria living at the O_2-H_2S interface must therefore always compete with the autocatalytic oxidation of sulphide. Both *Beggiatoa* and *Thiovulum* species have adapted to this requirement by growing as sheets at the transition between oxygen and sulphide. *Beggiatoa* cells, which are filamentous and gliding organisms, form thin mats over solid substrate from which H_2S is released

The chemical oxidation of H_2S in sea water and fresh water has been studied in the laboratory as well as in the chemocline of stratified water bodies The half life of sulphide is generally in the range of 1 to 3 hour at air saturation of oxygen and at 20^{o}C Jorgensen and Revsbech (1983) showed that the sulphide oxidation in the *Beggiatoa* mat was 10,000 to 100,000 fold faster than in water Also, diffusion calculations showed that formation of mats on solid substrates represented optimal

strategies for the bacteria to achieve a stable microenvironment, a high substrate supply, and an efficient competition with chemical sulphide oxidation The continuous gliding movement of *Beggiatoa* cells in mats are important for the availability of both O_2 and H_2S for the individual bacteria It seems most likely that the following pathway exists for inorganic oxidation of sulphur compounds (Buismen *et al.*, 1990).

$$HS^- \longrightarrow \text{membrane bound } [S^o] \longleftrightarrow S^o$$
$$\text{membrane bound } [S^o] \longrightarrow SO_3^{2-} \dashrightarrow SO_4^{2-}$$

The biological oxidation of sulphide to sulphate proceeds in two stages. In the first stage, which proceeds faster than the second stage, sulphide looses two electrons and membrane bound polymeric sulphur compounds are being formed. In the second step, this sulphur is oxidised to sulphite and then to sulphate. The following (biological) overall reactions occurs in an aerobic sulphide removal system

$$2HS^- + O_2 \longrightarrow 2S^o + 2OH^-$$

$$2S^o + 3O_2 \longrightarrow 2SO_4^{2-} + 2H^+$$

So far, little relevant information is available about the aerobic oxidation of sulphide into sulphur by the colourless sulphur bacteria It was shown that the products of the sulphide oxidation are elemental sulphur and sulphate and that the formation of these oxidation products proceeds independently of the pH of the reactor solution in the range 6.5 to 9.0.

The source of sulphides (H_2S, HS^-, S^{2-}) is generally from the reduction of sulphate by sulphate reducing bacteria (SRB). These bacteria are strict anaerobes which utilise a number of organic compounds as a source of carbon and energy such as lactate, acetate and ethanol These compounds are end products of the metabolism of fermentative heterotrophs and are readily available

in a consortium of bacteria in an anaerobic environment. Therefore, sulphate reducing bacteria are abiquitous to virtually any anaerobic environment conducive to microbial growth (Montogomery and McInerney, 1990)

Identification of Beggiatoa and SRB: *Beggiatoa* occurs in waters where both oxygen and H_2S are present. They may form mats with a slightly yellowish-white appearance due to deposition of internal sulphur globules. They generally are large and may be motile with a characteristic gliding movement They are 2 to 15 μm in diameter and may be upto 1,500 μm long, individual cells, if visible, are 4 to 16 μm long. Identification has been made on the basis of microscopic examination of the suspected material and by comparing the organisms with available photographs. The sulphate reducing bacteria, such as *Desulphovibrio*, cannot be identified by direct microscopic examination, but physiologically

Mechanism of Selfgranulation of Sludge (SGS)

Nelson *et al.* (1986) found that *Beggiatoa* glided to the place where oxygen concentration was less than 10 micromoles (0.32 mg/L) and sulphide concentration was also less than 10 micromoles (0.34 mg/L) in the sulphide gradient culture. This aspect of *Beggiatoa* lead them to the surface of SGS where oxygen and sulphide concentrations were suitable for them. It is suggested that the mechanism of SGS formation is one in which filamentous bacteria, *Beggiatoa*, wrap around anaerobic sludge, and interfere with the penetration of oxygen into the anaerobic sludge The symbiotic relationship between SRB and SOB makes the SGS stable The SRB-SOB symbiosis is schematically presented in Fig 2.

One of the interesting aspects of the MRB is the mechanism of self granulation of sludge. In the MRB, oxygen is supplied at the AV where few microorganisms are present. On the other hand, a significant amount of microorganisms remain in the BV, the DO supplied in the AV will be consumed rapidly if organic

substrates in the wastewater is high. Consequently, near anaerobic condition allowing the growth of sulphate reducing bacteria (SRB) are developed in the BV.

From the above mentioned observations, the mechanism of selfgranulation of sludge in the BVs is considered as follows.

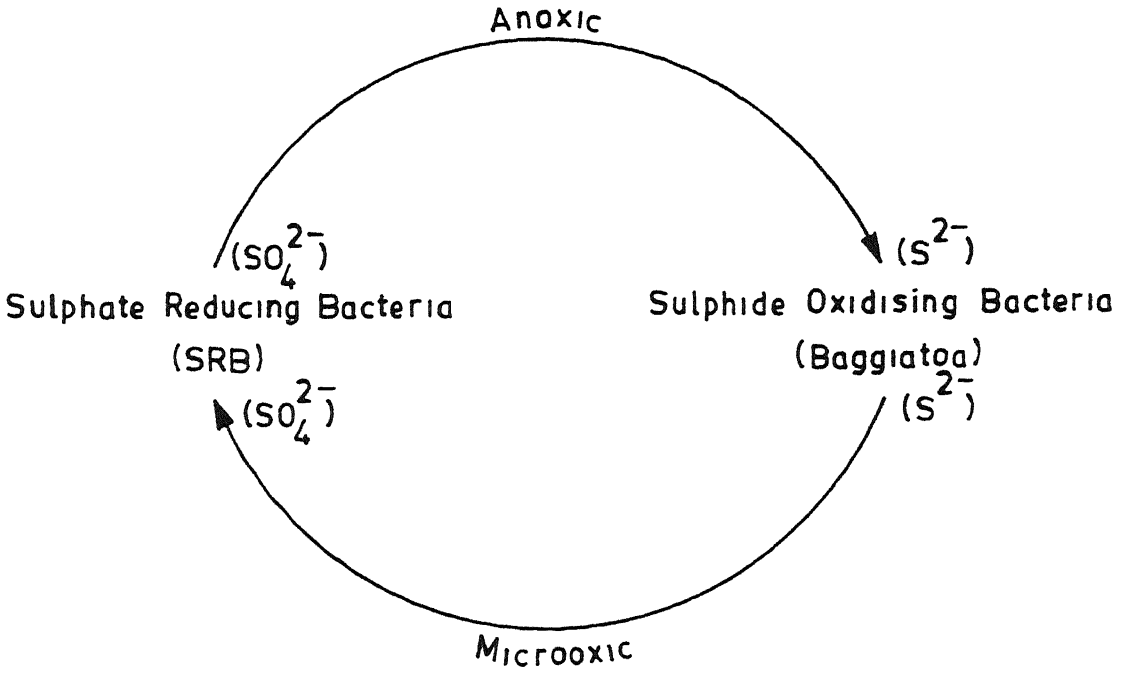


Fig 2 Sulphate Reducing—Sulphide Oxidising Bacterial Symbiosis.

Fig 3 explains interaction among the SRB. *Beggiatoa* as SOB and other anaerobic bacteria Organic substrate in the wastewater containing sulphate diffuse into the SGS and is hydrolysed to organic acids by anaerobic bacteria. These acids are then utilised by the SRB resulting in sulphate reduction and oxidation of

organic acids into carbon dioxide. Sulphide produced from this reaction diffuses to bulk liquid through SGS surface Though the oxygen supply is limited in the BV, there is a chance for the microorganisms on the SGS surface to come into contact with the oxygen Because the oxygen consumption rate of bacteria oxidising sulphide is much higher than that of the organic substrate oxidising bacteria, most of the oxygen is utilised by the sulphide oxidising bacteria which are microaerophilic. *Beggiatoa*, which is one of the most adapted bacteria for such an environment, increases on the SGS surface in a thin film manner

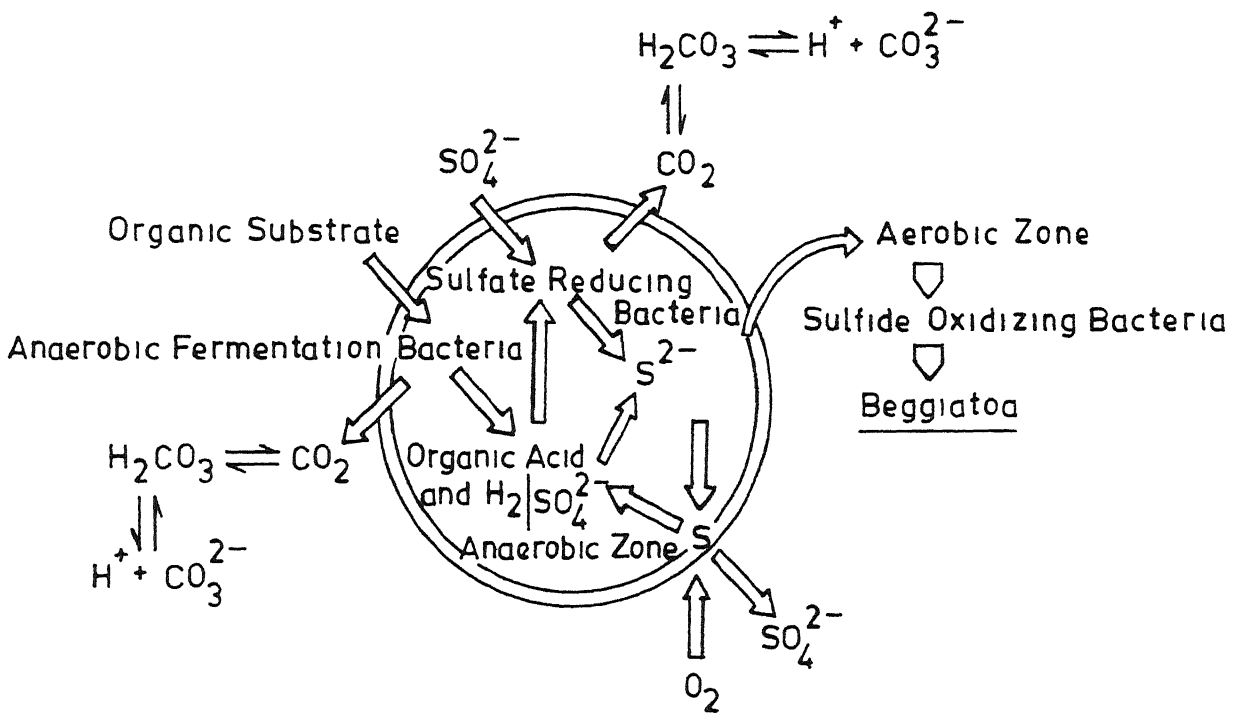


Fig. 3 Schematic of Bacterial Interaction in Self Granulated Sludge

Source : Takahashi and Kyosai (1988)

Background Work on MRB

Takahashi and Kyosai (1988) reported that for MRB process concentration and composition of sulphur compounds present in wastewater is quite important Eventhough high sulphate concentration is inhibitive to anaerobic process, it seems to be advantageous to MRB process Sulphides and sulphates are the key compounds for the symbiotic interaction existing in the MRB process.

MRB process has been first developed by Takahashi and Kyosai (1988) in Japan. They have used MRB consisting of five stages for the treatment of municipal primary effluent (soluble BOD = 50 mg/L) The SGS formed in BV was 2 to 10 mm diameter and could be maintained in BV with upflow velocity of 144 m/day. The effluent quality was as fine as that of conventional activated sludge process with HRT of 4.5 hours The average sulphate concentration of Municipal wastewater was 15 mg-S/L.

The earlier work done on MRB process in environmental engineering laboratory at the Indian Institute of Technology, Kanpur, India (Mansoor, 1991) is for the further treatment of anaerobic effluent (distillery waste) from Downflow Stationary Fixed Film (DSFF) reactor. In this study, three stage bench scale MRB system (capacity = 11 L) with an HRT of 8 hour was used and the MRB effluent quality was as good as that of ASP effluent Further, though there was good sludge accumulation, there was no SGS formation in BVs

SCOPE OF THE INVESTIGATION

Review of MRB process presented in earlier section clearly indicates that this process involves microbial population distinctly different from those employed in conventional (albiet modified) aerobic and anaerobic processes for wastewater

treatment. Limited studies carried out on this process reveal advantageous utilisation of symbiotic relationship between SRB and SOB in reducing organic content of wastewater. It is claimed that this process may be a good competitor to traditional aerobic-anaerobic processes for treatment of wastewater under several situations. Though the underlying microbial principles are fairly demonstrated, many aspects of the process require further investigation and it would take quite sometime before this technology can achieve the status of emerging technology and ultimately become the available treatment technology as defined by USEPA (1986). Following are some of the aspects which could be explored in the initial stages

1. Previous investigations have used excess sludge obtained from anaerobic treatment of wastewater as external seed to start MRB process and thereby achieve a significant growth of SRB and SOB. It would be of interest to know, if the required microbial population can be developed without any external seed.

2. In all biological processes, there are two ways of maintaining desired levels of biomass, i e., either recirculation of settled biomass after solid-liquid separation or retainment of biomass by immobilising the microbes. Earlier studies have not considered possibility of providing additional nonreactive surface for the development of necessary microbial growth. However, as demonstrated with conventional aerobic-anaerobic processes, there are definite advantages in several situations of providing external surface for retainment of biomass. From microbial requirement of MRB process it appears that providing external nonreactive surface in the BV would be a logical attempt to improve the process performance.

3 Sulphur is one of the key elements in the growth of SRB and SOB and it would be of interest to study the effect of presence of various sulphur species in the influent and the consumption of sulphur in relation to organic matter removal.

4 Since very limited investigations are carried out employing MRB process, there are several microbiological and engineering parameters which are yet to be studied. For example, the nutritional requirements, microbial kinetics, organic matter removal and excess sludge production, quantification of oxygen requirement, effect of environmental conditions such as pH and temperature, engineering aspects of the process, etc are to be studied

The present investigation mainly aims at investigating points raised in 1 and 2 above. Limited attempts are also made to clarify some of the issues raised in 3 and 4 above.

EXPERIMENTAL METHODOLOGY

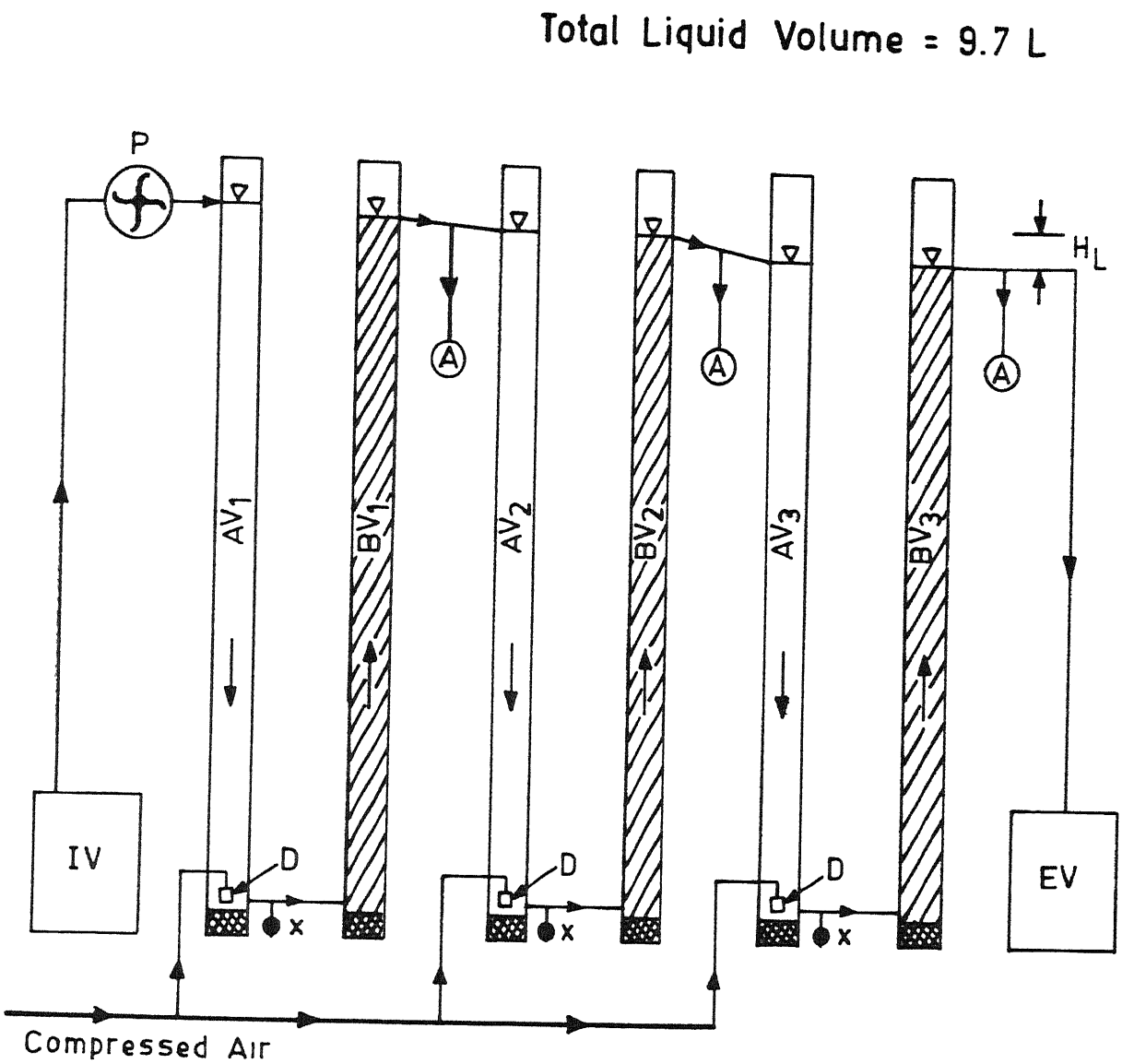
The experimental investigation was planned and carried out in two parts The first part involved bench scale model of MRB made on the lines used by previous investigators called as `MRB I' system The second part involved bench scale model of MRB in which BVs were provided with additional nonreactive surface for promoting growth of SRB-SOB microbial system called as `MRB II' system

Part I : MRB I System

The experimental bench scale model of this system was made up of three stages, each stage having one AV and one BV as shown in Fig 4. All aeration vessels were made of PVC columns having approximately 3.7 cm internal diameter and 180 cm height. All biological reaction vessels were made of plexiglass columns having the same dimensions as that of AV. Further details are presented in Table 1

Part II : MRB II System

The experimental bench scale model of this system was also made up of three stages, each stage having one AV and one BV



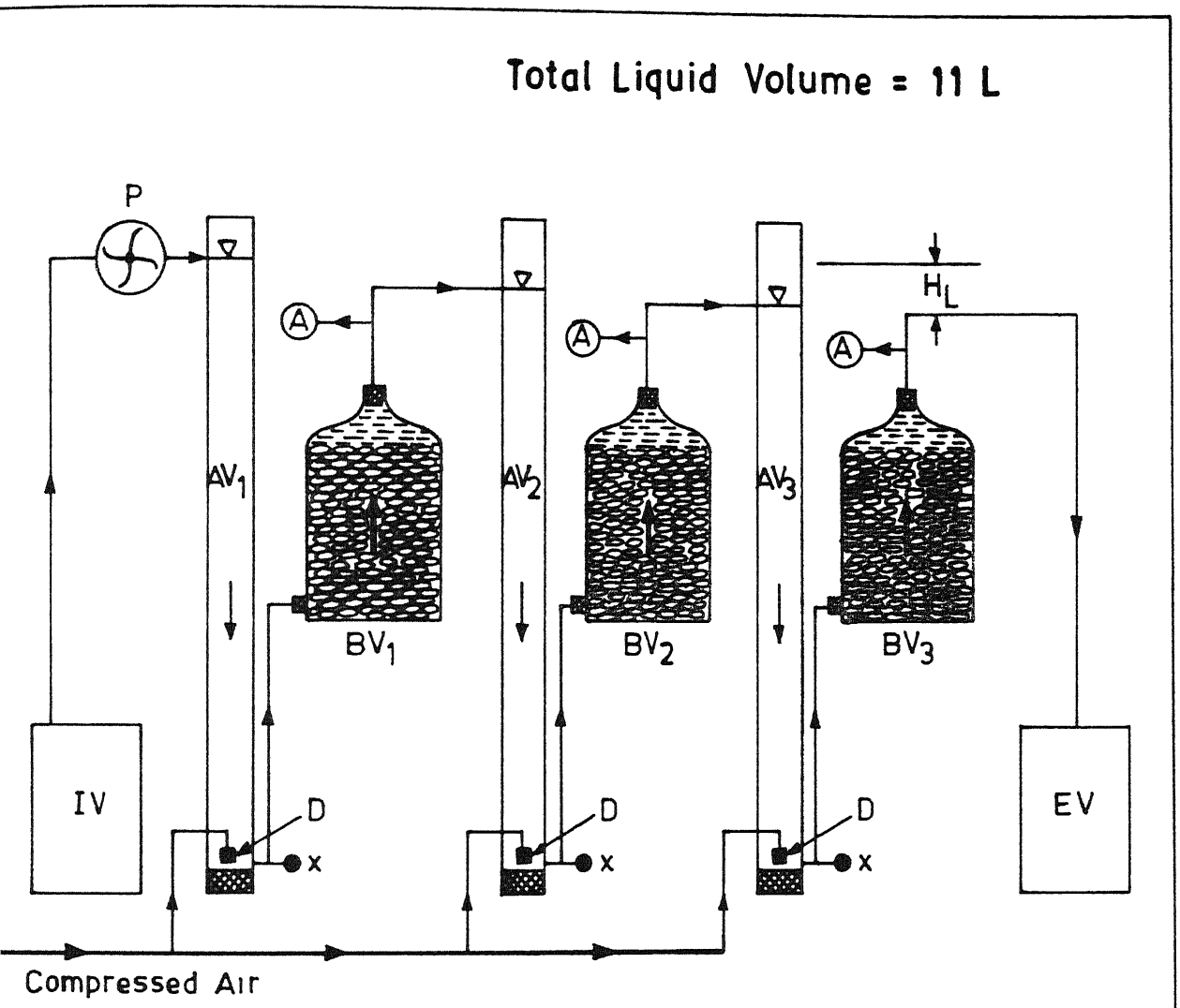
P Peristaltic Pump IV Influent Vessel EV . Effluent Vessel
x Draining Facility D . Diffuser A Sampling Port
AV Aeration Vessel BV Biological Reaction Vessel

Fig 4 Schematic of MRB I System.

as shown Fig 5 The first two AVs were made of plexiglass columns and the last AV was made of PVC column. All AVs were of

Table 1. MRB I and MRB II System Details

Parameter	MRB I System	MRB II System
AV Details		
Internal Diameter	3 7 cm	3.7 cm
Average Liquid Height	150 cm	150 cm
Average Liquid Volume	1 6 L	1.6 L
Flow Rate	1400 mL/hour	1570 mL/hour
Flow Velocity	31.3 m/day	35 m/day
HRT	1.16 h	1.02 h
BV Details.		
Internal Diameter	3.7 cm	11 2 cm
Average Liquid Height	150 cm	21 3 cm
Average Liquid Volume	1.6 L	2.1 L
Packing Material	Nil	PVC Rings (Length : 2.54 cm; OD : 1.8 cm, ID : 1.5 cm, Porosity : 0.86, Specific Surface Area :13.33 sq.cm/ cubic cm)
Flow Rate	1400 mL/hour	1570 mL/hour
Flow Velocity	31.3 m/day	3 8 m/day
HRT	1 16 hour	1.34 hour
Overall Details.		
Number of Stages	3	3
Total Liquid Volume	9 7 L	11.0 L
HRT	7 hour	7 hour



P Peristaltic Pump IV Influent Vessel EV Effluent Vessel
x Draining Facility D Diffuser A Sampling Ports
AV Aeration Vessel BV Biological Reaction Vessel

Fig. 5 Schematic of MRB II System.

approximately 3.7 cm internal diameter and 180 cm height. Respiratory bottles of about 2 L capacity were used as BVs. Packing media used for attached growth consisted of PVC rings having 2.5 cm length, and 1 8 and 1.5 cm outer and inner diameters respectively. Further details are presented in Table 1

Start up

MRB I system was started by filling all the system vessels with domestic wastewater whose general characteristics are shown in Table 2 Initially the contents of the entire system including influent container were recirculated internally at an HRT of 8 hour for 10 days prior to initiation of organic load application on a continuous basis

Table 2 Characteristics of Domestic Wastewater*

Parameter	Value
pH	7.4 - 7 6
Turbidity, NTU	32 - 55
Total hardness, mg/L as $CaCO_3$	330
Ca^{2+} hardness, mg/L as $CaCO_3$	300
Acidity, mg/L as $CaCO_3$	90
Alkalinity, mg/L as $CaCO_3$	360
Specific conductivity, mhos/cm	$26\ 5 \times 10^{-4}$
Total phosphate, mg/L as P	5.0
COD, mg/L	150 - 180

* Adopted from Goel (1983)

For MRB II system, first all the biological reaction vessels with packing media were kept in an anaerobic condition to form sufficient anaerobic slime layer over the packing media by filling with diluted solution of sugar cane molasses prepared in Indian Institute of Technology, Kanpur campus domestic wastewater

(Table 2) These vessels were sealed for about 70 days Then the system was started by filling all the aeration vessels and influent container with the same domestic wastewater and internal circulation at an HRT of 6 hour for 10 days prior to application of organic load (diluted molasses) on a continuous basis In both cases contents of the influent containers were replaced with fresh domestic wastewater at an interval of 3 days

Continuous Operation

Both systems were operated at a fixed HRT of 7 hour. Thus the flow velocity in all the vessels of MRB I system was 31 3 m/day while in MRB II system all AVs had a flow velocity of 35 0 m/day and all BVs had an upflow velocity of 3 8 m/day. The aeration vessels of both systems were drained intermittently whenever substantial suspended growth was observed in the vessels. This was done to minimise contribution of organic removal in all AVs as hypothesized in the development of MRB systems Continuous operation of both systems were carried out for about 125 days (over 4 months) A summary of operating schedule is presented in Table 3

Performance Evaluation

The development and performance evaluation of MRB systems were carried out under ambient temperature by estimating chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, sulphates, sulphides and dissolved oxygen (DO). The DO was monitored in all vessels COD, pH, temperature and DO were monitored daily. The stagewise performance evaluation of each MRB system was started after 26 days of continuous operation Overall performance of the two systems was evaluated by estimating COD, BOD, pH, sulphate and sulphide of the samples withdrawn from influent and effluent containers once in a day, prior to changing the contents of these containers The identification of microbial population developed in various vessels was done using optical microscope and comparing with available information.

Table 3 Summary of Operational Schedule

Sl. No.	Days After Initiation of Organic Load Appln.	Feed Description	Av. Inf. COD mg/L	SO_4^{2-} added* mg/L	Nutrients added
1	1 - 10	Domestic Wastewater	150	Nil	Nil
2	11 - 17	Diluted sugar cane molasses with IITK tap water & 10% addition of domestic wastewater	275	Nil	Nil
3	18 - 23	Diluted sugar cane molasses with IITK tap water	275	60	Nil
4	24 - 74	"	150	60	Nil
5	75 - 106	"	200	60	Nil
6	107 - 109	Glucose solution prepared in IITK tap water	210	60	Yes**
7	110 - 115	No feeding			
8	115 - 118	Glucose solution prepared in IITK tap water	210	60	Yes**
9	119 - 125	Glucose solution prepared in IITK tap water	437	Nil	Yes**

* This is over and above that was present in sugar cane molasses and dilution water

** Urea and phosphate to maintain COD:N:P of influent wastewater as 100:5.1

Analytical Techniques

Different analytical techniques (Table 4) were employed for the estimation of various parameters of influent and effluent samples collected during the experimental studies. All techniques used were of routine type and hence are not described here.

Table 4 Analytical Techniques Employed

Parameter	Instrument/Technique	Reference
pH	Systronics pH Meter	-
Temperatue	Thermometer	-
COD	Potassium Dichromate, Closed Reflux Method	Standard Methods (APHA, 1985)
BOD	Incubation at 35° C for 5 days	"
Sulphate	Gravimetric Method	"
Sulphide	Iodometric Method	"
DO	DO Meter 141 Systronics	-
Identificaton of microbes	Optical Microscope	Standard Methods (APHA, 1985)

RESULTS AND DISCUSSION

The major emphasis in this investigation has been on (1) development of microbial system which utilises symbiotic relationship between sulphur reducing and sulphur oxidising bacteria as hypothesized by Takahashi and Kyosai (1988) for degradation of organic matter present in wastewater without any external seeding, and (2) comparative evaluation of two types of MRB systems (MRB I and MRB II) which simulate conditions corresponding to reactor configuration described as upflow sludge blanket and stationary fixed film Accordingly two bench scale models as described in previous sections were set up. MRB I system was designed to achieve self granulation of sludge (SGS) in biological vessels while MRB II was designed to achieve favourable environmental conditions for development of microbial population which works on symbiotic relationship of SRB and SOB. Since conditions for formation of SGS are not well-understood and it is generally believed that SGS formation is difficult to achieve

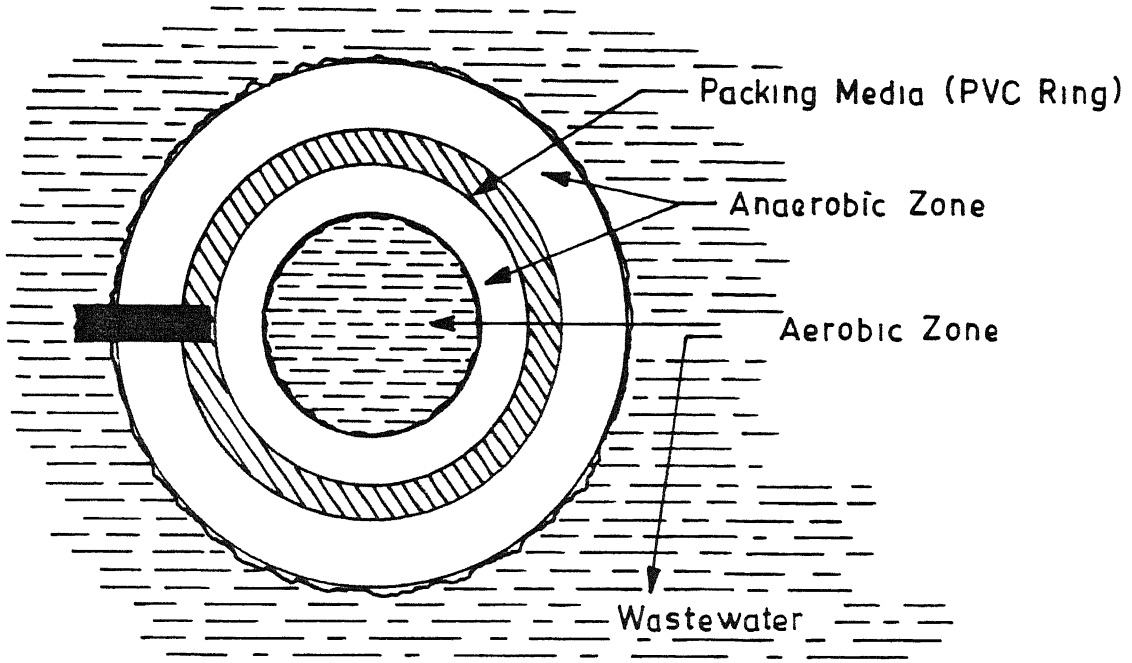
(Mansoor, 1991 and Basu, 1991), efforts were made to study development of the same in MRB I system MRB II which provided additional nonreactive surface for microbial growth was thought to be a logical modification of MRB system reported (Takahashi and Kyosai, 1988) from the experience of adopting similar modifications in conventional aerobic and anaerobic processes Conditions required for symbiotic relationship of SRB and SOB can also be promoted in MRB II system. The proposed microbial interactions in this system is shown in Fig. 6.

Visual and Microscopic Observations

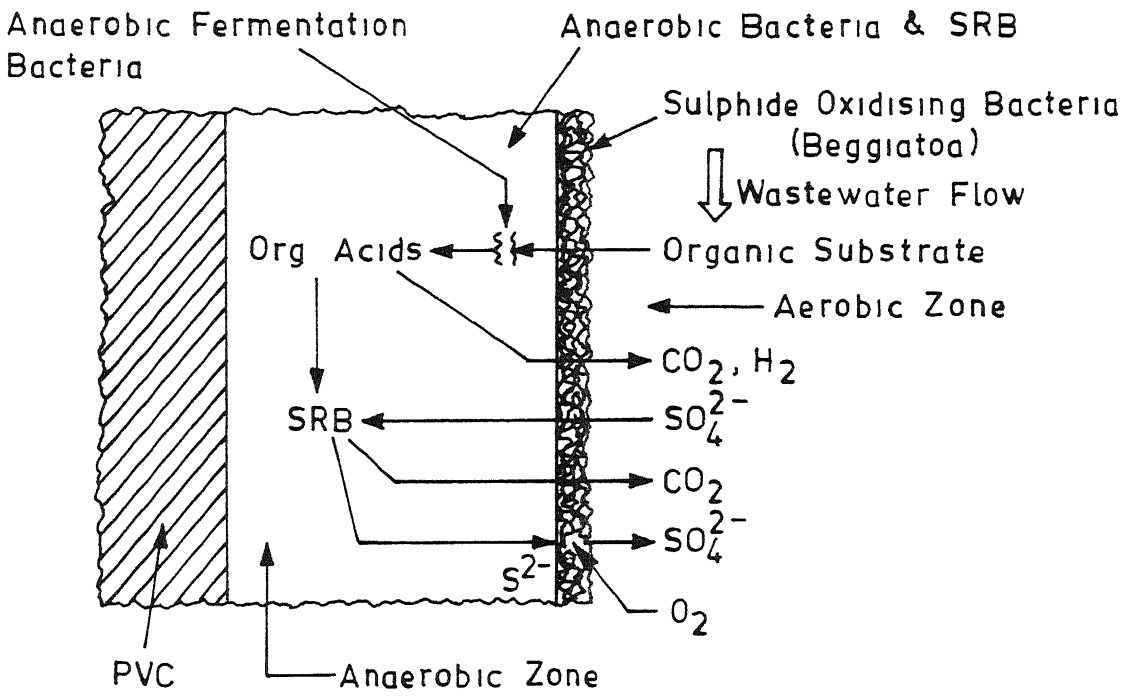
MRB I and MRB II systems were operated for a period of 4 months as per the schedule presented in Table 3. Since it is difficult to perform tests which would demonstrate gradual build up of desired microbial population, careful visual observations were made which ultimately lead to the confirmation of the presence of SRB and SOB in both systems. A brief summary of observations is presented as follows

1. After 1 week of continuous operation of organic load, bacterial colonies appeared on the walls of all biological vessels of MRB I The number of colonies was more in the first BV and least in third BV.

2. Dispersed microbial growth developed in all AVs. Extent of growth decreased gradually from first AV to third AV Whenever dispersed growth developed to a considerable extent as noticed from COD reduction, the AVs were drained to avoid contribution of AVs in COD reduction as hypothesized while developing MRB system (Takahashi and Kyosai, 1988) During the initial stages of study, the frequency of draining was once in 4 days and at the later stage the same reduced to once in a fortnight This observation supported the hypothesis that as more and more biomass accumulated in BVs, the contribution of AVs is insignificant for COD reduction



Cross Section of PVC Ring with Immobilized Biofilm



Enlarged View of Blackened Portion

Fig 6 Proposed Bacterial Interaction in Immobilized Biofilm

This hypothesis is further justified by the observation in the last stages of the study, when the organic matter was easily degradable (glucose), there was no need of draining, that is there was insignificant accumulation of biomass in AVs

3. After 2 weeks of continuous operation, the bacterial colonies were grown to a larger size (about 3 mm) and was attached to the wall of BVs of MRB I. After about 45 days of continuous operation, BV1 was completely covered with wall growth and turned blackish. Similar phenomenon was observed, but with a lag in BV2 and BV3

4. After 16 days of continuous operation, whitish to pale yellow spots appeared on the wall of BVs of MRB I and on the surface of packing media of BV1 and BV2 of MRB II After 30 days of continuous operation, these spots disappeared in BV1 and BV2 but appeared in BV3 of MRB II

5. After 2 months of continuous operation, it was observed that a portion of BV1 of MRB II turned whitish to pale yellow. This colouration of a portion of BV1 disappeared after 3 days This phenomenon repeated at irregular intervals in the same location as well as in BV2 and BV3 but with a lag. It is hypothesized that this colouration may be due to deposition of sulphur granule by the sulphide oxidising bacteria (Jorgensen and Revsbech, 1983)

6. Microscopic observations on a random sample of biomass withdrawn from BVs of MRB I and MRB II after about 2-3 months confirmed the presence of SOB Subsequently from all BVs of both systems, biomass was withdrawn at regular intervals and kept in 100 mL beakers Within 1 to 2 hours self granulation of sludge

was observed which covered the bulk of biomass with white-pale yellow films surrounding it. The microscopic view of such a biomass is shown in Fig.7. The filamentous type bacteria could be identified as *Beggiatoa* by comparing with available microscopic photographs and by observing their gliding mobility through microscope.

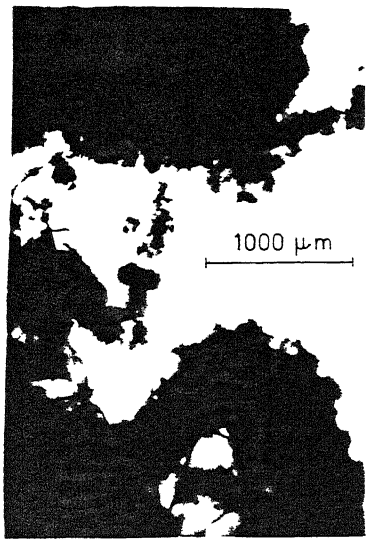
Performance Evaluation

Performance of both MRB systems was evaluated through monitoring several influent, effluent and system parameters. The system parameters included pH, temperature and dissolved oxygen levels The significant influent and effluent parameters included chemical oxygen demand, biochemical oxygen demand and sulphate and sulphide concentration. Overall performance of systems as well as of each stage of MRB I and MRB II were monitored through estimation of these parameters as per preplanned schedule. The results are presented in Figs 8-9 and Table 5. These figures also represent the variation in operational conditions as reported in Table 3 Dissolved oxygen levels monitored in all vessels of both systems are presented in Table 6 In general following remarks can be made from these results

- Both systems show more or less similar performance Change in the applied BOD/COD load did not significantly affect the removal of these parameters Appreciable BOD/COD removal occurred during the entire period of operation. Since measures were taken to eliminate dispersed growth and hence contribution to organic matter removal in aeration vessels, it can be said that the reduction in BOD/COD occurred through utilisation of symbiotic relationship between SRB and SOB as hypothesized

- Stagewise reduction of organic matter occurred in accordance with the visual observations of the extent of growth in the biological vessels.

Source Takahashi and Kyosai (1988)



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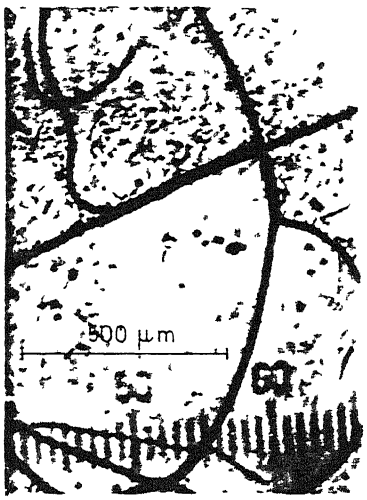

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Fig 7 Typical Microscopic View of the Portion of Biomass in SGS at Various Magnification Levels

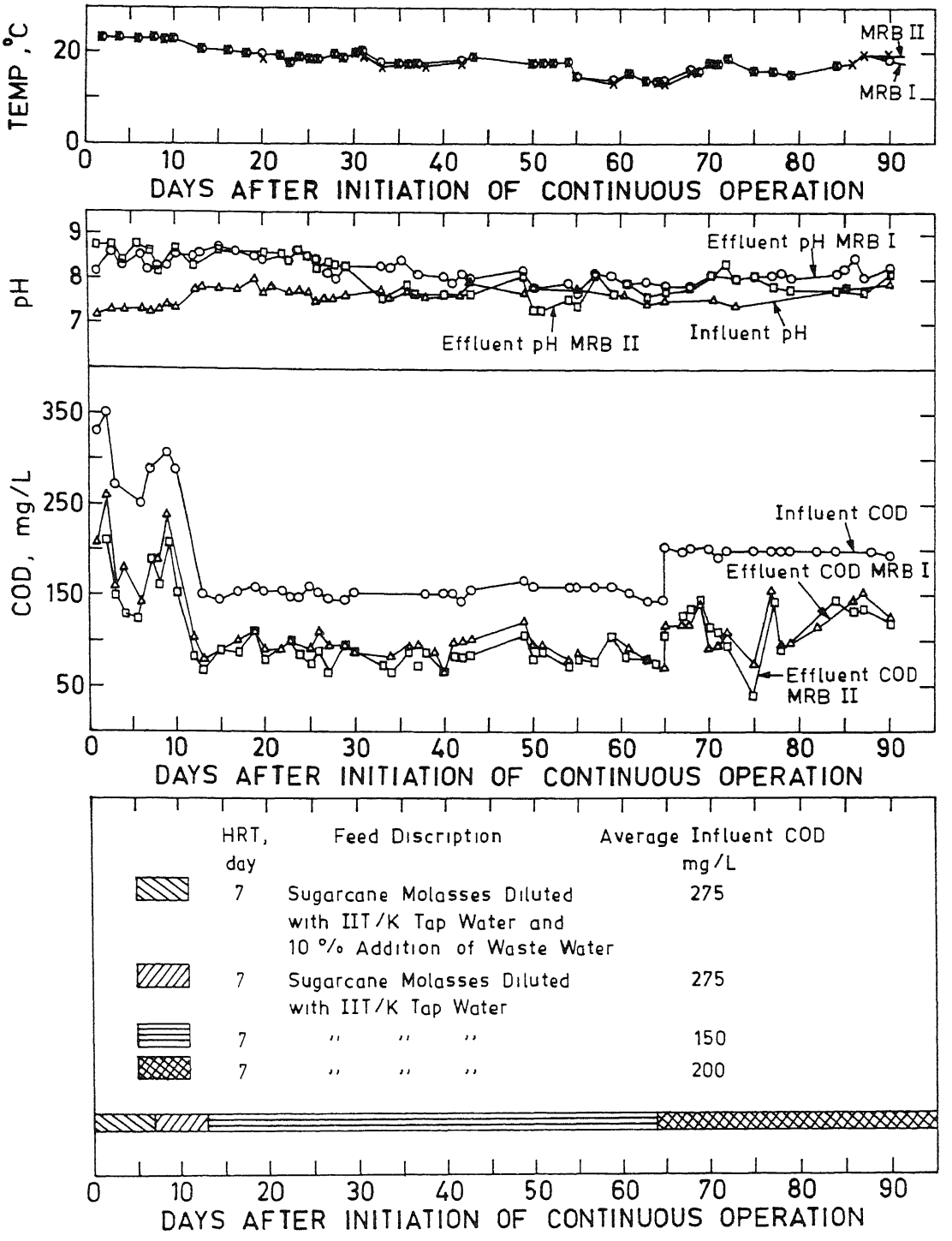

Fig 8 The Overall Performance History of MRB Systems

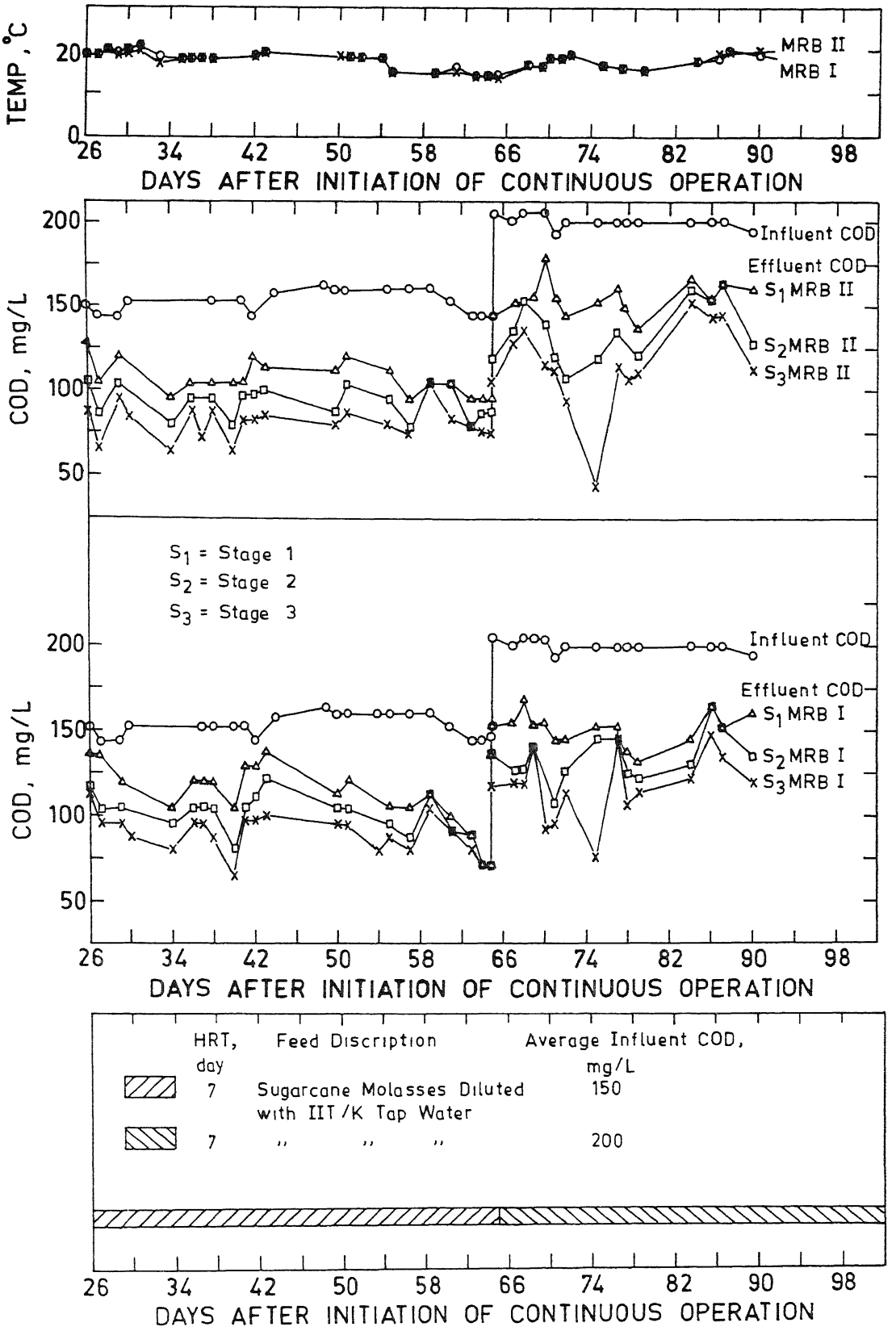

Fig 9 Stagewise Performance History of MRB Systems

Table 5. Overall Peformance History of MRB Systems.

Sl No	Days After Initiation of Organic Load Appln	Feed Type	System	Influent Parameters				Effluent Parameters			
				COD mg/L	BOD mg/L	SO_4^{2-} mg/L	S^{2-} mg S/L	% Removal COD	% Removal BOD	SO_4^{2-} mg/L	S^{2-} mg S/L
1	1 - 12	Diluted sugar cane molasses	MRB I	275±58 (7)				31 3 (9)			
			MRBII	275±58 (7)				38 2 (9)			
2	13 - 64	-do-	MRB I	150±7 4 (28)	56±5 (9)			40 (31)	71 4 (9)		
			MRBII	150±7 4 (28)	56±5 (9)			46 7 (31)	66 1 (9)		
3	65 - 96	-do-	MRB I	200±6 8 (15)	87±4 8 (16)	173±25 8 (4)	2 2± 8 (6)	45 (16)	72 4 (16)	169±13 4 (4)	1 2± 4 (7)
			MRBII	200±6 8 (15)	87±4 8 (16)	173±25 8 (4)	2 2± 8 (6)	44 4 (16)	55 2 (16)	149±15 2 (4)	2 6±1 8 (7)
4	97 - 99	Glucose Solution	MRB I	210±14 (2)	170±13 (3)			88 6 (2)	82 4 (3)		
			MRBII	210±14 (2)	170±13 (3)			91 0 (2)	87 1 (3)		
5	100-105	No Feeding	-	-	-	-	-	-	-	-	
6	106-108	Glucose Solution	MRB I	210±14 (2)	168±0 (2)			85 45 (2)	81 25 (2)		
			MRBII	210±14 (2)	168±0 (2)			89 0 (2)	92 86 (2)		
7	109-115	-do-	MRB I	437±3 8 (6)	336±7 (2)		5±1 7 (2)	72 5 (6)	56 4 (2)		1 4± 8 (2)
			MRBII	437±3 8 (6)	336±7 (2)		5±1 7 (2)	74 1 (6)	57 2 (2)		6 4±1 2 (2)

Numbers in paranthesis indicate number of observation included in calculation of average and 95% confidence range

Table 6. DO Levels in MRB Systems.

Sl No	Days After Initiation of Organic Load Application	System	DO, mg/L					
			AV1	BV1	AV2	BV2	AV3	BV3
1	1 - 24	MRB I	5 9±1 8 (9)	4.8±2.4 (9)	5.7±2.6 (9)	4.8±3 (9)	6±1.8 (9)	4.9±2.9 (9)
		MRBII	5 9±1 4 (9)	3.7±2.7 (9)	5.7±2 (9)	3 5±2 4 (9)	6.2±1.6 (9)	3.8±2.8 (9)
2	25 - 35	MRB I	4±2 6 (8)	3.4±2 3 (8)	5 6±1.4 (8)	4.6±1 7 (8)	4.9±2.3 (8)	4.3±2.1 (8)
		MRBII	3.4±1.9 (8)	2.1±.8 (8)	3.8±2.8 (8)	2.5±1.7 (8)	5.2±.6 (8)	2.3±.9 (8)
3	36 - 55	MRB I	6 5±2.3 (9)	5±1.5 (9)	6.5±.6 (9)	4.9±1.4 (9)	5.1±1.2 (9)	3.8±.9 (9)
		MRBII	3.6±.8 (9)	2.2±.9 (9)	3.5±2.5 (9)	2.6±1.5 (9)	4.3±1 (9)	2.4±1.3 (9)
4	56 - 85	MRB I	4.4±1 (14)	3.4±1 (14)	4.8±1.5 (14)	3.8±1.5 (14)	4.5±2.3 (14)	3.6±1.9 (14)
		MRBII	3 8±1.6 (14)	2.8±.7 (14)	4.6±2.2 (14)	2.9± 6 (14)	3.9±.4 (14)	2.3±.5 (14)
5	86 -115	MRB I	2.7± 6 (8)	2±.3 (8)	2.3±1.1 (8)	2.1±.8 (8)	2.3±.9 (8)	1.9±.9 (8)
		MRBII	3.7±1 5 (8)	2 8±1.1 (8)	3.4±1 6 (8)	2.4±.9 (8)	3.2±1.1 (8)	2.0±1.1 (8)

Numbers in paranthesis indicate number of observations included in calculation of average and 95% confidence range

- No definite observation could be made about requirement and removal of sulphate since influent level of the same was quite high (approximately 150 mg/L) and the method of estimation adopted is not very sensitive to properly reflect minor changes. However, it can be said that the sulphate consumption is insignificant.

- Sulphide concentration in the effluent is very low.

- pH of influent and effluent of both systems varied in the range of 7 2 to 8.8. Buisman *et al.* (1990) reported that sulphide oxidation proceeds independently of the pH in the range of 6.5 to 9.0. This indicates that hydrogen ion concentration was at the optimum level for sulphide oxidation.

- For any biological system, temperature plays an important role In the present investigation, studies were conducted at ambient temperatures In general, there was significant variation of daily temperature and also the ambient temperature was quite low compared to the optimum temperature. Higher removal rates and rapid development of required levels of microbial population could have been achieved at high temperatures (30-35^{o}C)

- Dissolved oxygen levels maintained in both systems are comparable with those reported and required for achieving desired environmental conditions for symbiotic action of SRB and SOB

CONCLUSIONS

Based on the results of the present investigation and nthesis of the results presented in literature, following nclusions may be drawn

1 It is possible to develop self granulated sludge and microbial population involving sulphate reducing and sulphide oxidising bacteria, without any external seeding Domestic wastewater can serve as a source of these organisms.

2 Immobilised growth conditions and suspended growth conditions in biological vessels yield similar results in terms of organic matter removal efficiencies.

3. MRB system efficiency as obtained in the present investigation appears to be comparable to that of the activated sludge process as reported in literature Considering the merits of the MRB process, it appears to be a good alternative to conventional aerobic processes

4 Consumption of sulphate appears to be insignificant and hence several types of wastewater could be treated using MRB system.

SUGGESTIONS FOR FUTURE WORK

The following suggestions are made for the future research as a logical continuation of the work presented in this thesis.

1 The bench scale model used for MRB II system used in the present study could not achieve proper plug flow conditions in the biological vessels. It is felt that if hydraulics of vessels is appropriately modified to promote better plug flow conditions, then substantial improvement in the performance can be expected.

2 It is necessary to study factors which affect self granulation of the sludge as well as the optimum conditions required to promote the growth of SRB and SOB

3 In order to further develop this process it is necessary to quantify factors such as maximum growth rate, nutrient

requirements, yield coefficient, etc. of the microbial population involved

4 Eventhough it appears that the energy requirement of MRB process is lower than that of the conventional (albiet modified) aerobic processes, it is necessary to quantify the energy requirement of this process for realistic comparison.

5 Studies are necessary to suggest the potential waste types which could be advantageously treated by MRB.

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